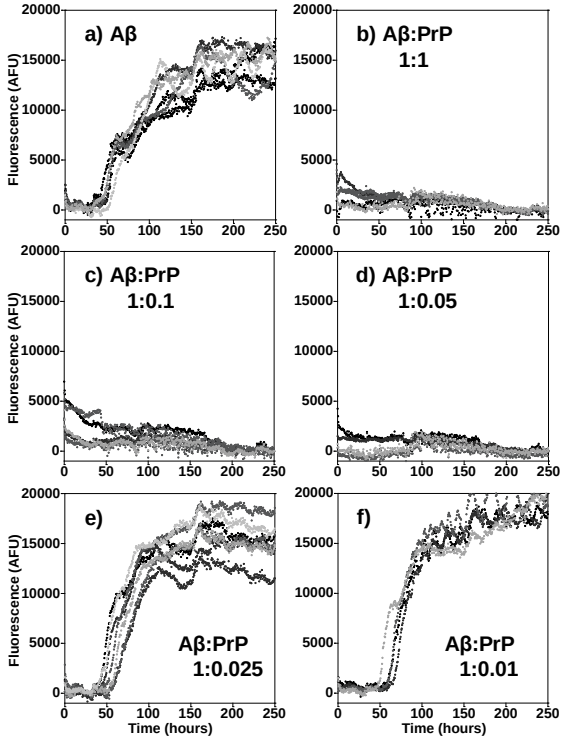

Figure 1: PrP^{C} inhibits Aβ_{40} fiber formation.
The kinetics of Aβ_{40} fiber formation was monitored by fluorescence upon ThT binding to amyloid. Aβ_{40} alone (a), and in the presence of: 1 mole equivalent of PrP(23-231) (b); 0.1 (c); 0.05 (d); 0.025 (e); and 0.01 mole equivalents of PrP(23-231) (f). Aβ_{40} monomer (10 µM) was incubated at pH 7.4 in HEPES buffer (30 mM), NaCl (160 mM), at 30 ºC with intermittent agitation. As little as 500 nM of PrP(23-231) completely inhibits Aβ_{40} fiber formation over 250 hours.

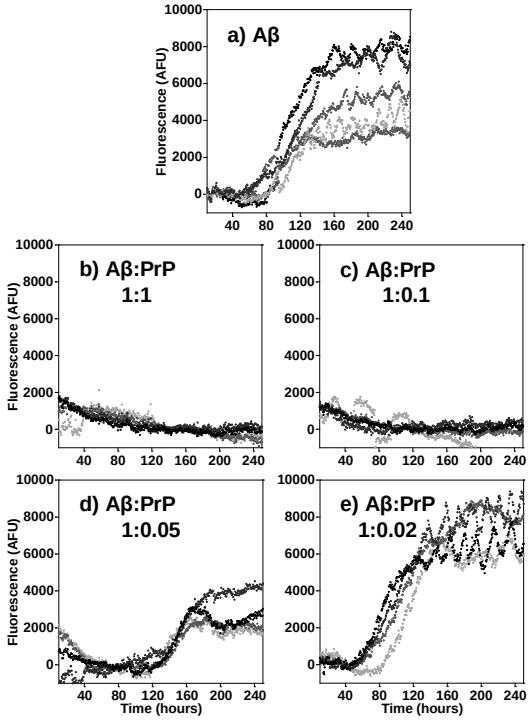

Figure 2: PrP^{C} inhibits Aβ_{42} Fiber Formation.
The kinetics of Aβ_{42} (5 µM) fiber formation was monitored by fluorescence upon ThT binding to amyloid. Aβ_{42} alone (a), and in presence of: 1 mole equivalents of PrP(23-231) (b); 0.1 (c); 0.05 (d), and 0.02 mole equivalent PrP(23-231) (e). Aβ_{42} monomer (5 µM) was incubated at pH 7.4 in HEPES buffer, 160 mM NaCl, at 30 ºC with intermittent agitation. As little as one twentieth PrP(23-231) will inhibits Aβ_{42} fiber formation over 250 hours.

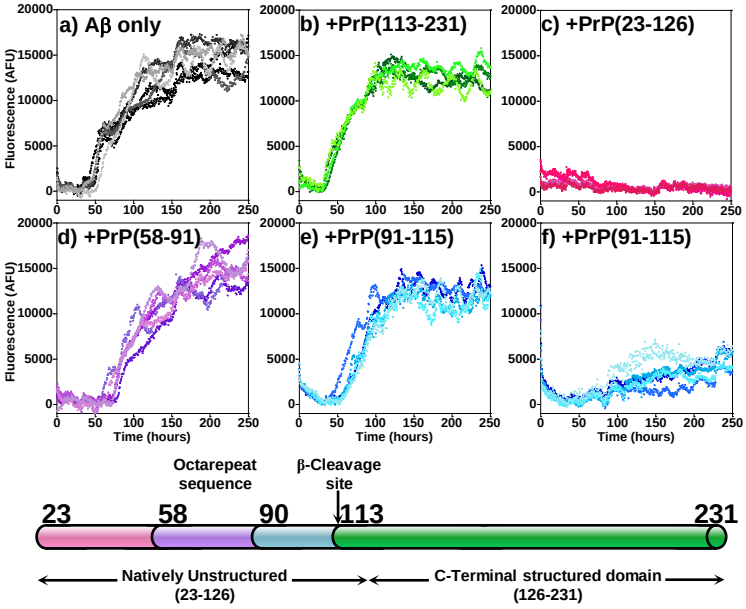

Figure 3: Aβ Fiber Formation in the Presence of PrP^C Fragments

The kinetics of $A\beta_{40}$ (10 µM) fiber formation was monitored by fluorescence upon ThT binding to amyloid. a) $A\beta_{40}$ alone; b) 1 mole equivalent of PrP(113-231); c) 0.1 mole equivalents of PrP(23-126); d) 0.1 mole equivalents of PrP(58-91); e) 0.1 mole equivalents of PrP(91-115); f) 1 mole equivalent of PrP(91-115). $A\beta_{40}$ was incubated at pH 7.4 in 30 mM HEPES buffer, 160 mM NaCl, at 30 ºC with intermittent agitation.

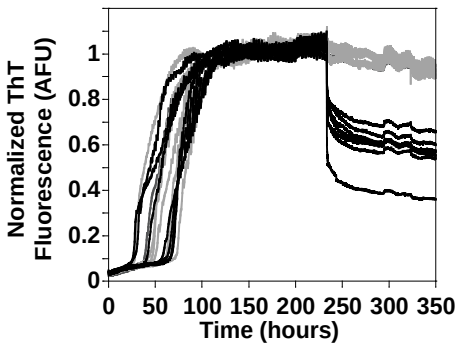

Figure 4: PrP^C Induced Aβ Fiber Disassembly

Mature $A\beta_{40}$ fibers alone (red) are formed over a 234 hour period, after which 1 mole equivalent of PrP(23-231) is added to 6 of the reaction wells (black). $A\beta_{40}$ (10 µM) samples were incubated at pH 7.4 in 30 mM HEPES buffer, 160 mM NaCl, at 30 ºC with intermittent agitation. Addition of PrP^C caused a 40 % reduction in ThT fluorescence within 90 minutes.

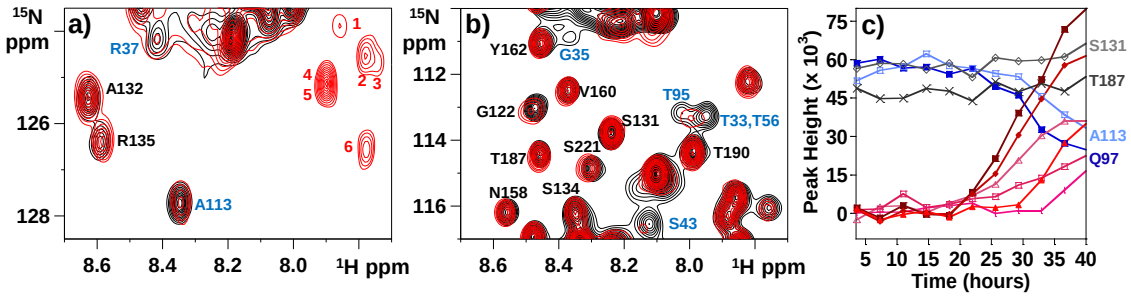

Figure 5: ^{15}N HSQC NMR of the PrP(23-231) binding to Aβ_{40} Oligomer
Selected regions of 2D ^{15}N-^{1}H HSQC of PrP(23-231) alone (black) and PrP(23-231) with 1 mole equivalent of Aβ_{40} after a 40 hour incubation (red). Amide resonances that show a marked loss of signal are labelled in blue. c) Peak intensity plotted against time, six new peaks are observed after 20 hours (various shades of red) and the reduction in the intensities of; A113 and Q98, while S131 and T187 remain unaffected over 40 hours. Spectra obtained at 30 ºC in 50 mM phosphate buffer at pH 6.5, 50 µM of PrP^{C} and Aβ.

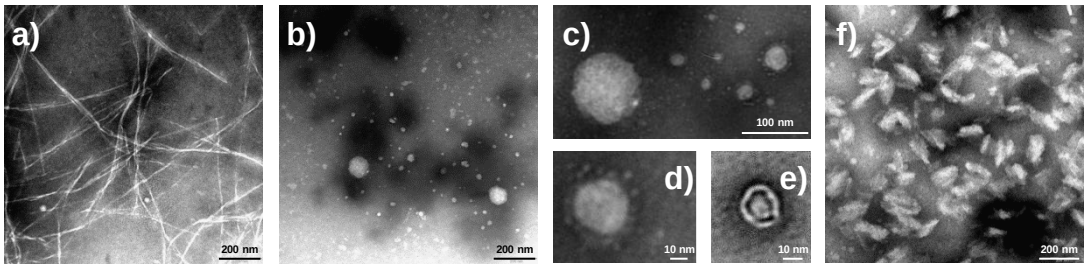

Figure 6: TEM of the Aβ in the Presence of PrP^C
Negative stain transmission electron images of (a) $A\beta_{40}$ fibers alone. Aβ incubated with PrP(23-231) (b-e). (f) PrP^C added to mature $A\beta_{40}$ fibers. $A\beta_{40}$ (10 µM) samples were incubated at pH 7.4 in 30 mM HEPES, with 160 mM NaCl at 30 °C with intermittent agitation for 300 hours. The TEM grids were negatively stained using phosphotungstic acid. Only Aβ oligomers are observed where Aβ is incubated with PrP^C (0.1 mole equivalents).

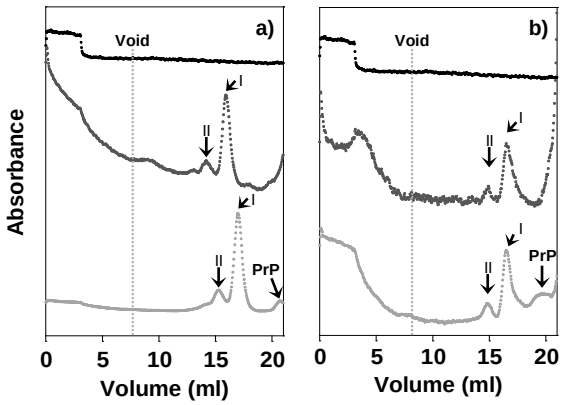

Figure 7: Size Exclusion Chromatography of $A\beta_{40}$ Oligomer in the presence of PrP^C
$A\beta_{40}$ incubated with PrP(23-231) panel-a and PrP(23-126) panel-b. $A\beta_{40}$ only (top), with 0.5 µM PrP (middle) and with 2 µM of PrP (bottom). Oligomers I and II indicates the complexes formed at ~60 and ~100 kDa, respectively. $A\beta_{40}$ (10 µM) with/without PrP samples were incubated at 30 ºC and using 30 mM HEPES at pH 7.4, 160 mM NaCl and with agitation for 250 hours. The size exclusion chromatography was carried out at 4 ºC and pH 7.4, using a Superdex 200 column.

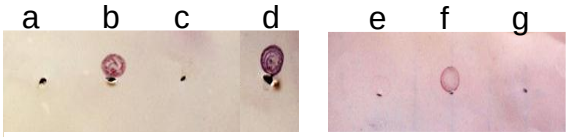

Figure 8: Aβ Oligomer Antibody binding dot blot assay.
a) $A\beta_{42}$ essentially monomer, b) Incubated $A\beta_{42}$ 30 µM with PrP(23-231) 10 µM , c) $A\beta_{42}$ fibers, d) disassembled $A\beta_{42}$ fibers with 1.5 mole equivalents of PrP(23-231), e) PrP(23-126) 10 µM only, f) $A\beta_{40}$ + 0.1 mole equivalents of PrP(23-231) and g) 10 µM PrP(23-231) only, were dotted on a membrane and examined using antibody (A11) which is sensitive to oligomers but not to fibers or monomers.

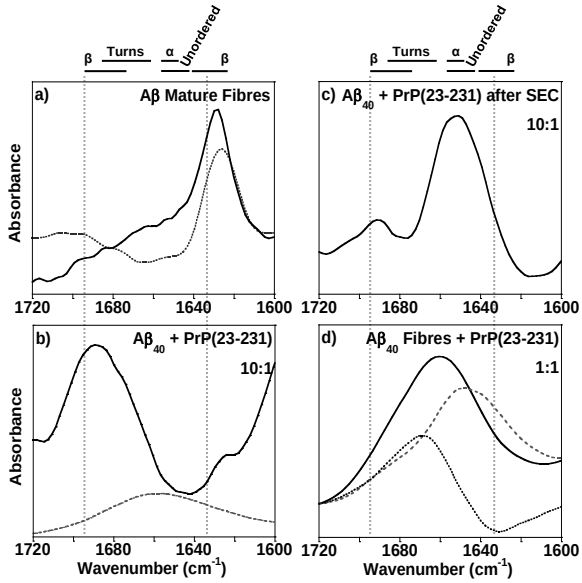

Figure 9: IR Spectra of Aβ Oligomers in the Presence of PrP^C

Structural characterization using IR amide-I band of a) $A\beta_{40}$ mature fibers (dashed) $A\beta_{42}$ mature fibers (solid). b) $A\beta_{40}$ monomer incubated with 0.1 mole equivalents of PrP(23-231) (solid black), PrP(23-231) alone (dashed grey). c) $A\beta_{40}$ with PrP^C oligomers eluted from Size Exclusion Chromatography (SEC). d) $A\beta_{40}$ mature fibers with 1 mole equivalent of PrP(23-231) (solid black), PrP(23-231) alone (dashed grey) and difference spectra (dotted). $A\beta_{40}$ monomer (10 μM) was incubated at pH 7.4 using 30 mM HEPES buffer, 160 mM NaCl at 30 ºC. The dotted lines highlight 1695 and 1633 cm^{-1}. $A\beta_{40}$ oligomer formed in the presence of PrP^C shows an increase in 1695 cm^{-1} amide-I band.

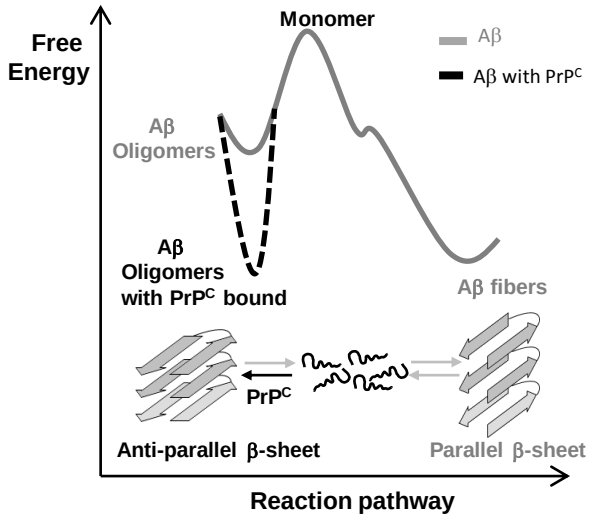

Figure 10: PrP^C Stabilizes the Oligomeric form of Aβ

The binding of PrP^C stops the transition from anti-parallel to parallel sheet necessary for a transition from oligomer to fiber. PrP^C may stop the rotation of the β-sheet pairing from intra- to intermolecular. The energy barrier to go from anti- parallel to parallel arrangement of β-sheet is likely to be very large. It is therefore more likely that anti-parallel oligoermic arrangement largely disassemble to form the in-register parallel sheets formed in fibers. Whether the transition from anti-parallel oligomer to fibers is on or off-pathway it is clear the binding of PrP^C stabilizes the oligomeric form.